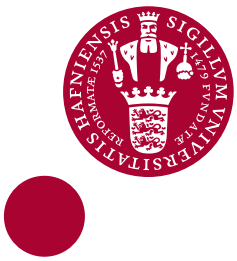

Lenalidomide plus bendamustine-rituximab does not overcome the adverse impact of TP53 mutations in mantle cell lymphoma

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TP53 mutations are associated with significant poorer outcomes in mantle cell lymphoma (MCL), and new treatment strategies are highly warranted. Lenalidomide has shown high efficacy in MCL; however, there is no knowledge of its effect on *TP53* mutated cases. In this study, we show that the addition of lenalidomide to bendamustin-rituximab does not overcome the adverse impact of *TP53* mutations.

The outcome of MCL has been improved markedly during the past decades; however, the course of the disease remains highly heterogeneous.[1–3] Several biomarkers have been proposed to stratify patients at diagnosis, i.e., morphologic subtype, proliferation index and the MCL international prognostic index (MIPI), but so far none has been systematically implemented in treatment stratification.[4,5]

TP53 aberrations are associated with more aggressive disease and poorer outcome.[6,7] In a recently published study by the Nordic Lymphoma Group (NLG) of younger patients receiving intensive, cytarabine-containing therapy and autologous stem-cell transplantation (ASCT), we showed that *TP53* mutations signified a subgroup of patients with exceedingly poor outcome, overruling all other known prognostic markers.[8] In addition, Aukema *et al.* recently reported similar findings based on p53 protein expression by immunohistochemistry.[9] Thus, alternative therapeutic strategies are highly warranted in this subset of patients.

In chronic lymphocytic leukemia (CLL), lenalidomide maintenance has shown promising response rates in high-risk patients, including patients with *TP53*-aberrations.[10] The Nordic MCL4 trial, Lena-Berit (*clinicaltrials.gov identifier 00963534*), investigated the additive effect of lenalidomide to bendamustin-rituximab (LBR) in elderly/frail patients.[11] In general, this regimen was associated with an unexpected high frequency of toxic events, especially infections, cutaneous events and secondary malignancies; however, the patient cohort may still serve to investigate the effect of lenalidomide-addition to chemo-immunotherapy in subsets of patients. Thus, in this current study, we investigated the outcomes of patients from the Nordic MCL4 trial in relation to common MCL-related, genetic aberrations, with a special emphasis on the *TP53*-mutated cases.

An elaborate description of methods are presented in the *Online Supplementary Data* and, furthermore, specific details on the MCL4 trial and genetic analyses are described in Albertsson-Lindblad *et al.*[11] and Eskelund *et al.*,[8] respectively. In brief, 50 patients, >65 years or ≤65 years and unfit for ASCT, were enrolled between 2009 and 2013. The regimen consisted of an induction phase (weeks 1-24) of six cycles of LBR followed by a maintenance phase of lenalidomide (weeks 25-56) (*Online Supplementary Figure S1*).[11] Pretreatment DNA samples [(39 bone marrow (BM) and 7 peripheral blood (PB)] were selected by availability. MCL was detected in all samples by either flow cytometry or by a positive MRD marker. Mutational analysis with targeted NGS was performed on eight MCL-related genes: *ATM, KMT2D, CCND1, TP53, WHSC1, BIRC3, NOTCH1* and *NOTCH2*; and droplet digital Polymerase Chain Reaction (ddPCR) was used to identify two commonly deleted gene regions, chr17p13 (*TP53*) and chr9p21 (*CDKN2A*).[8] The study was performed in agreement with the Declaration of Helsinki and was conducted according to the guidelines for Good Clinical Practice, issued by The International Conference on Harmonization (ICH). The protocol was approved by all national Ethical Review Boards. All patients signed a written informed consent to participate and to donate/provide samples from peripheral blood, bone marrow and tissue for biologic studies. The clinical trial was registered at *www.ClinicalTrials.gov* (*clinicaltrials.gov identifier 00963534*).

Patient characteristics are shown in *Online Supplementary Table S1*. In an extended clinical follow up (median follow up of 47 months compared to 31 months in our previous report), median overall survival (OS) was 69 months (95% CI 60.4-77.5; n events=23) and median progression-free survival (PFS) was 42 months (95% CI 28.5-55.5; n events=30) (Figure 1 A-B). Median time to progression/relapse was 53 months (95% CI 34.1-71.9) (Figure 1 C). None of the curves showed any sign of a plateau. At the current update, three additional cases of second primary malignancies (SPM) have been reported, making the total number of patients with SPM 9 (18%) (non-invasive skin cancers not included).

Baseline DNA samples were available for 46 patients (39 BM and 7 PB samples). Two samples did not reach sufficient quality for sequencing, and were only included in the deletion analyses. *TP53* deletions were detected in 9 (20%) patients and *CDKN2A* deletions in 10 (22%) patients. Five (11%) patients had both deletions. The most frequently mutated genes were *ATM*, detected in 15 (34%) patients, *KMT2D* in 8 (18%) and *TP53* in 6 (14%) patients (Figure 2, *Online Supplementary Table S2*). Thus,

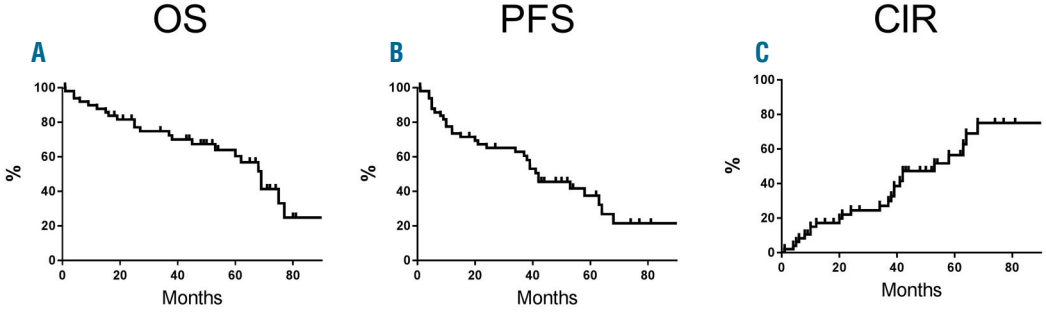

Figure 1. Kaplan-Meier estimates of all patients in the MCL4 trial. Kaplan-Meier plots for (A-C) all patients by intention-to-treat (n=50). OS: overall survival; PFS:progression-free survival; CIR: cumulative incidence of relapsing or progressive disease.

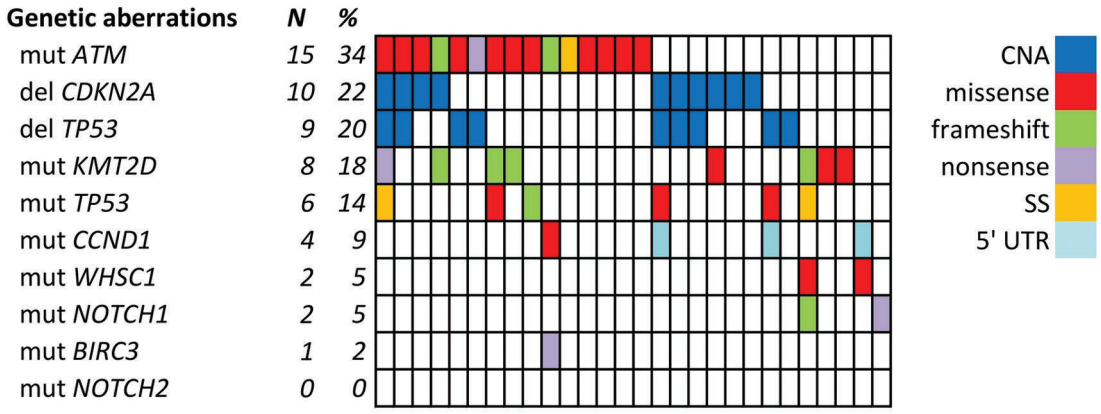

Figure 2. Overview of genetic aberrations. Overview of genetic landscape for all patients with detected genetic aberrations (n=28). Each row represents a gene, and each column represents a patient. Colour coding: Dark blue: Copy number alteration, (CNA); Red: missense mutations; Green: frameshift indels; Violet: nonsense mutations; Orange: splice-site mutations; Light blue: Mutations in the 5 untranslated region (UTR).

the prevalence of all genetic aberrations was comparable to our recently published study of younger MCL patients.[8]

Median OS for the *TP53* mutated and unmutated patients were 25 months (95% CI: 6.6-43.4) and 69 months (95% CI: 67.0-70.7), respectively (P<0.0001), median PFS were 10 months (95% CI: 0-22.9) and 42 months (95% CI: 21.8-62.2), respectively (P=0.001), and median time to progression or relapse were 10 months (95% CI: 0-22.9) and 58 months (95% CI: 35.7-80.3), respectively (P<0.0001) (Figure 3 A-C). One of the *TP53* mutated patients withdrew consent at day 28 and did not provide permission for further follow up and was hence censored at this time point. Of the remaining 5 patients, all progressed or relapsed during the study and none were alive at the current follow up. At the end of the induction phase, 3 *TP53* mutated patients were responding, but, of these, 2 patients had progressive disease at next follow up which was only 1.5 months later during the lenalidomide maintenance phase. All five patients had available MRD markers, but none achieved MRD negativity in both BM and PB at any time during follow-up evaluation.

Collectively, we show that *TP53* mutations retain very poor prognostic value despite the addition of lenalidomide to chemo-immunotherapy. Our findings are in contrast to preclinical models on lenalidomide, showing activity in CLL cell lines, independent of functional status of p53.[12] Furthermore, a clinical study on CLL has suggested activity of lenalidomide maintenance in *TP53*-aberrated patients, albeit so far only reported for PFS.[10] Ruan *et al*. showed promising response rates of L-R in MCL; however, data on *TP53* status was not included.[13]

A limitation to our study is the high number of treatment terminations related to toxicity. However, among the 5 *TP53* mutated patients available for follow up, only two patients withdrew due to adverse events (after receiving 7 and 11 cycles of lenalidomide, respectively, and while still being MRD positive), whereas the other 3 patients withdrew due to progressive disease (PD). Thus, we believe that our results do reflect the actual lack of efficacy of lenalidomide in these patients. Obviously, another drawback is the small cohort size, and thus the results will need validation in a larger cohort. Nonetheless, the results certainly argue against lenalidomide as the solution to the adverse impact of *TP53* mutations.

Interestingly, the only *TP53* mutated patient who had a long-lasting response (41 months) harboured a splice-site mutation which is rare for *TP53* (2.4% according to the IARC *TP53* Database). This sort of mutation may cause only loss-of-function, rather than dominant negative and oncogenic effects.[14] Another patient had a splice-site *TP53* mutation as well, but was lost to follow up due to withdrawal of consent at day 28.

Deletions of *TP53* and *CDKN2A* both showed trends towards inferior outcomes (*Online Supplementary Figure S2*). None of the other mutations analyzed in this study were associated with impact on outcome (*data not shown*). Thus, the combined results are similar to the findings in our recent report on younger MCL patients,[8] that the impact of *TP53* mutations overrules other genetic aberrations. The two deleted regions showed significant association to outcome in univariate models in our previous report, but only borderline significance in this present study. Most likely, this is only a reflection of the smaller patient cohort, rather than a biologic effect.

A total of 12 patients had a mutation and/or deletion of *TP53*, and they displayed significantly poorer outcome, with a median OS of 25 months (95% CI: 0-57.4, P=0.065), PFS of 12 months (95% CI: 6.6-17, P=0.016), and 50% of the patients had progressed/relapsed at 34 months (95% CI: 0.2-67, P=0.031) (Figure 3 D-F).

These data supplement the recently published results from the Nordic Philemon trial (ibrutinib, lenalidomide and rituximab) which showed almost similar outcomes of *TP53*-mutated and -unmutated patients,[15] thus suggesting that the effect of the Philemon regimen on *TP53* mutated cases is primarily exerted by ibrutinib, rather than by lenalidomide (or by the combined action of the two). Fortunately, novel frontline trials including Ibrutinib are ongoing (e.g., the European Triangle trial for younger patients and ENRICH trial for elderly) and will hopefully elaborate on this issue.

In conclusion, our study shows that the addition of lenalidomide to rituximab-bendamustine does not overcome the negative impact of *TP53* mutations. Thus, *TP53* mutated MCL remains a major challenge, and our results underline the importance of molecular profiling, including *TP53* status, in future trials exploring novel agents.

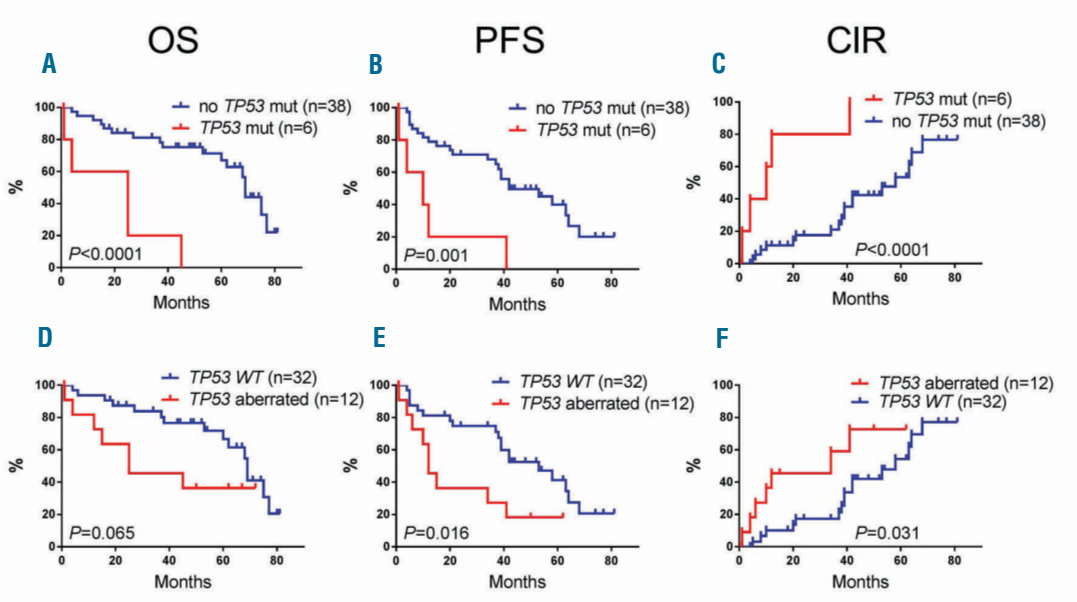

Figure 3. Kaplan-Meier estimates of MCL4 patients according to *TP53* aberrations. Kaplan-Meier plots for all patients with available DNA (A-C) according to presence or absence of *TP53* mutations, and (D-F) according to *TP53* aberrations (mutations and deletions) or *TP53* wildtype (WT). OS: overall survival; PFS: progression-free survival; CIR: cumulative incidence of relapsing or progressive disease.

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